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DOI: [https://doi.org/10.1016/0035-9203\(95\)90034-9](https://doi.org/10.1016/0035-9203(95)90034-9)

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-155527>

Journal Article

Published Version

Originally published at:

Skalsky, J A ; Joller-Jemelka, H I ; Bianchi, L ; Knoblauch, M (1995). Liver pathology in rural south-west Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 89(4):411-414.

DOI: [https://doi.org/10.1016/0035-9203\(95\)90034-9](https://doi.org/10.1016/0035-9203(95)90034-9)

## Liver pathology in rural south-west Cameroon

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### Abstract

In a prospective study, 102 hospital patients with liver disease were evaluated in West Cameroon, Africa. Blood donors, pregnant women and patients without liver disease served as controls. A total of 757 individuals were tested for markers of hepatitis A, B, C and D and for immunological markers (autoantibodies, procollagen III,  $\alpha$ -foetoprotein, CA50 antigen,  $\alpha$ -1-antitrypsin and antibodies to human immunodeficiency virus types 1 and 2). One-third of the liver disease patients had focal lesions on ultrasound examination. Histologically, 20 cases of cirrhosis, 14 cases of chronic hepatitis, 15 hepatocellular carcinomas and 17 cases of acute hepatitis were detected. All hepatic patients and virtually all controls had had a previous hepatitis A virus infection. Over 85% of adult patients and controls had at least one marker of hepatitis B virus infection. Over 30% of patients with liver disease had markers of possible hepatitis B virus replication. Anti-hepatitis C virus antibody was present in 18% of hepatic patients and in 6% of controls. Hepatitis C virus infection seems to play an important role in the development of chronic liver pathology; 40% of cirrhotic patients had a combined hepatitis B and C virus infection. Serum autoantibodies were frequently found and were not correlated with the presence of autoimmune liver disease.

**Keywords:** liver disease, hepatitis A, B, C, D, hepatocellular carcinoma, cirrhosis, Cameroon

### Introduction

Data on liver pathology and hepatitis virus serology from rural tropical Africa are scarce. Being impressed by the frequency of liver diseases in patients at the Manyemen General Hospital in the South-West Province of Cameroon, we conducted a prospective study to elucidate the nature, aetiology and diagnostic management of liver disease in hospital patients during a period of 2 years.

The study included a standardized clinical investigation, ultrasound examination, liver function tests, liver histology, hepatitis virus and human immunodeficiency virus (HIV) serology, and the determination of several autoantibodies and immunological markers. Blood donors, pregnant women and patients with minor complaints not related to the liver served as controls.

Manyemen is situated in the rural, tropical forest area of the South-West Province of Cameroon. The Presbyterian General Hospital at Manyemen has 115 beds and a large primary health care service and serves a population of about 250 000 in a area of 15 000 km<sup>2</sup>. Falciparum malaria and filarial infections are hyperendemic, whereas parasites invading the liver, such as *Schistosoma mansoni* and liver flukes, are rare.

### Study design and Methods

We investigated 102 consecutive patients from September 1990 to August 1992. Inclusion criteria were clinically suspected liver disease on grounds of jaundice, ascites, right upper quadrant pain or a pathological liver mass. Initially 135 patients were enrolled, but 33 had to be excluded because of incomplete data, erroneous diagnosis or pathological conditions not permitting liver biopsy.

For the history, clinical examination and abdominal ultrasound standardized questionnaires and record forms were used. Ultrasound examination was performed using a Siemens Sonoline® 1300 linear scanner of 3 MHz. Liver biopsy was either performed 'blindly' according to the Menghini technique or under laparoscopic control. The biopsy specimens were kept in 10% formalin and later examined by one of us (L.B.).

Basic laboratory determinations were performed according to the World Health Organization recommendations (WHO, 1980); liver function tests were performed with the aid of a Boehringer Reflotron-Photometer®.

For immunological investigations, sera were stored at –4°C and later analysed at the Section of Clinical Immunology of the University Hospital, Zurich, Switzerland.

Markers of hepatitis A, B, C and D viruses (HAV, HBV, HCV, HDV) and autoantibodies such as anti-nuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), and anti-smooth muscle antibodies (ASM) were determined by indirect immunofluorescence, anti-M2 antibodies (AM2) by an anti-M2 enzyme-linked immunosorbent assay (ELISA) (Elias, Freiburg, Germany), procollagen III by P III-peptide radio-immunoassay (RIA) (Boehringer, Marburg, Germany), and  $\alpha$ -1-antitrypsin with the aid of a BNA-Nephelometer-Analyzer® (Boehringer, Marburg, Germany). HIV-1 and HIV-2 antibodies were also investigated, using commercial screening test kits and following the procedures recommended by the manufacturers (Abbott Laboratories, North Chicago, Illinois, USA). HIV-1 and HIV-2 antibodies were determined with the Abbott recombinant HIV-1 and HIV-2 enzyme immunoassay. Confirmation was by Western blotting for HIV-1 (Du Pont, USA) and HIV-2 (Pasteur, France). Anti-HCV was measured by a second generation ELISA (Ortho Diagnostic), positive results being confirmed by the Chiron recombinant immunoblot assay second generation HCV test (Chiron Corporation, USA).

### Results

The 102 patients with liver disease consisted of 67 males and 35 females, age range 6 months to 80 years (mean 40.5 ± 19 years); 64 of 95 gave a history of regular alcohol intake (mainly industrial beers and local palm wine), 7 of 64 reported a blood transfusion, 24 of 60 reported a previous episode of jaundice, and 41 of 53 had taken traditional herbal medicines.

#### Clinical presentation

The 5 most frequently encountered clinical presentations among 102 patients were jaundice (related to liver disease), 31; right upper quadrant pains, 20; ascites, 17; right upper quadrant tumour, 16; and hepatomegaly, 15.

#### Abdominal ultrasound examination

Focal lesions such as abscesses, tumors or metastases were found in 34 of the 98 patients examined. Sterile, chocolate-coloured pus could be aspirated in 5 instances. Differentiation between amoebic liver abscess, hepatocellular carcinoma (HCC) and malignant lymphoma or metastases was difficult. Puncture and aspiration with a 0.9 mm × 70 mm Luer needle was safe and helpful in these cases; no complication occurred.

#### Liver function tests

Liver function tests were performed on 93 patients

with liver disease.  $\gamma$ -Glutamyltransferase ( $\gamma$ -glutamyl-transpeptidase, EC 2.3.2.2) was elevated in 67%, aspartate aminotransferase (glutamic-oxalacetic transaminase, EC 2.6.1.1) in 57% and alanine aminotransferase (glutamic-pyruvic transaminase, EC 2.6.1.2) in 45%.

Bleeding and coagulation times, determined by the Duke/Lee and White methods (WHO, 1980) were normal in all except 3 patients, in whom there was no histological proof of severe, diffuse liver disease.

#### Histology

Histological diagnoses were made for 98 patients (Table 1). Of the 20 patients with liver cirrhosis, 8 had a

**Table 1. Histological diagnoses for 98 hospital patients in Cameroon<sup>a</sup>**

Liver cirrhosis	20
Macronodular	3
Micronodular	5
Unspecified	12
Acute hepatitis	17
Viral	2
Unspecified	2
Non-specific reactive	11
Special forms (giant cell)	2
Hepatocellular carcinoma	15
Chronic hepatitis	14
Chronic active hepatitis	7
Chronic persistent hepatitis	3
Granulomatous hepatitis	4
Secondary biliary inflammation	9
Liver fibrosis	8
Amoebic liver abscess	7
Alcoholic liver disease	6
Tropical splenomegaly syndrome	6
Others	14
Burkitt's lymphoma	3
Metastatic liver disease (adenocarcinoma)	2
Haemosiderosis	2
Liver anomalies (Caroli syndrome, cysts)	2
Fatty liver	2
Inconclusive histological diagnosis	2
HELLP syndrome (combined diagnosis)	1

<sup>a</sup>Occasionally 2 diagnoses were recorded for one patient.

<sup>b</sup>Steatosis as the main histological finding.

concomitant HCC, and 7 patients had HCC in a non-cirrhotic liver. There was a positive correlation between concomitant cirrhosis and HCC ( $\chi^2=11.82$ ;  $P=0.00059$ ) (Table 2). Malaria pigment was seen in nearly all the histological specimens.

**Table 2. Association between hepatocellular carcinoma and liver cirrhosis**

	Hepatocellular carcinoma	
	No. of patients	Present Absent
Liver cirrhosis		
Present	20	8 12
Absent	78	7 71
Total	98	15 83

#### Serological markers of viral hepatitis and HIV

The results are summarized in Table 3 and the Figure. All patients with liver disease and virtually all controls had had a previous infection with HAV; 25% of the healthy children up to 1 year old, and 90% of those up to 15 years old, who were tested, had anti-HAV antibodies. About 90% of all the liver disease patients, blood donors and pregnant women had a marker of HBV infection.

Significantly more pregnant women and blood donors were positive for anti-hepatitis B surface antigen anti-

**Table 3. Prevalence of serum markers of viral hepatitis, HIV and syphilis in different populations**

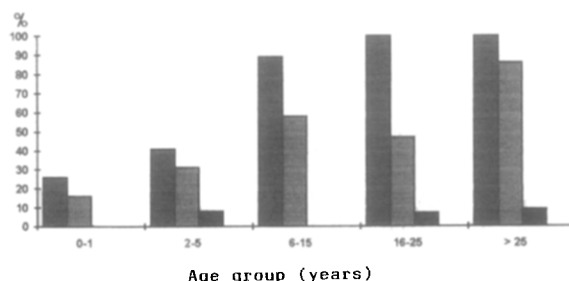
	Healthy pregnant women	Blood donors	Patients with minor complaints	Patients with HIV-1	Patients with liver disease
Total no.	383	85	108	93	88
Females	383	41	52	73	35
Males	—	44	56	20	53
Age (years) <sup>a</sup>	17 (17-48)	33 (17-55)	16.9 (0.5-80)	34 (2-61)	40.6 (0.5-80)
Percentages with the following markers <sup>b</sup>					
HAV (IgG)	98.8	96.4	77.7	97.5	100.0
HAV (IgM)	0.8	0	nd	0	4.0
HBsAg	5.4	9.8	6.5	9.1	31.4
Anti-HBs	61.0	55.8	38.0	58.2	32.6
Anti-HBc (IgG)	87.4	90.1	54.6	86.3	93.6
Anti-HBc only	nd	nd	nd	nd	31.3
HBeAg	nd	nd	nd	nd	18.5
Anti-HBe	nd	nd	nd	nd	35.2
HCV (IgG)	6.1	6.4	4.7	5.4	18.4
HDV	0	0	0	nd	2.3
HIV-1	2.9	2.3	1.8	100	2.3
HIV-2	0	0	0	0	0
RPR <sup>c</sup>	17.0	nd	nd	nd	nd

<sup>a</sup>Mean (range in parentheses).

<sup>b</sup>nd = Not determined.

<sup>c</sup>Rapid plasma reagin test for syphilis; other abbreviations re defined in the text.

body (anti-HBs) than patients, the converse being true for the antigen itself (HBsAg). About 10% of children up to 5 years old were anti-HBs positive, as were about 40% of those aged 15 years. The corresponding figures for anti-hepatitis B core antigen (anti-HBc) immunoglobulin G (IgG) were even higher, 30% and 57% respectively.



**Figure.** Age distribution of hepatitis markers in hospital patients with minor complaints unrelated to liver disease, Cameroon, 1990-1992. Dark grey bars: anti-hepatitis A IgG; light grey bars: anti-hepatitis B core antigen IgG; black bars: anti-hepatitis C IgG.

Among patients with liver disease, markers of possible HBV replication were positive in a high proportion: HbsAg in 31%, hepatitis B e-antigen (HBeAg) in 18%, and anti-HBc (alone) in 31%.

Anti-HDV was found in only 2% of patients with liver disease, but anti-HCV was significantly more common in such patients than in controls (18% versus 6%;  $P<0.025$ ). Because of the inclusion of children, patients with minor complaints not related to liver disease had a somewhat lower infection rate with HAV, HBV and HCV than the control patients, including the prevalence of HBsAg and anti-HBs.

The prevalence rate of anti-HIV-1 among both controls and liver disease patients was low, averaging 2%.

#### Immunological markers

The results are summarized in Tables 4 and 5. Procollagen III was elevated in approximately 75% of young people, 63% of blood donors and 53% of liver disease patients. CA50 antigen was elevated in more liver disease patients than in blood donors, but was also present in 38% of children up to 5 years old. Anti-nuclear antibodies (ANA) were found in 75% of liver patients but also in 25-35% of controls and youngsters. In 13 liver disease patients the titres were  $>1:320$ . AMA and AM2 were found in 22% of liver patients, the corresponding figures for blood donors being 5% and 16% respectively. In none of these patients with ANA or AMA could a

**Table 4. Prevalence of immunological markers in patients with minor complaints, not related to liver disease in different age groups**

Age group (years)	0-1	2-5	6-15	16-25	>25
Total no.	19	13	26	15	35
Females	14	3	13	8	13
Males	5	10	13	7	22
Mean age (years)	0.8	3.6	9.6	20.3	34.7
Patients with the following markers <sup>a</sup>					
HAV (IgG/IgM)	5 (26.3%)	6 (41.2%)	23 (88.5%)	15 (100%)	35 (100%)
HBsAg	1 (5.3%)	2 (15.4%)	1 (3.8%)	0 (-)	3 (8.6%)
Anti-HBs	2 (10.5%)	1 (7.7%)	10 (38.5%)	10 (66.7%)	18 (51.4%)
Anti-HBc IgG	3 (15.9%)	4 (30.8%)	15 (57.7%)	7 (46.7%)	30 (85.7%)
HCV (IgG)	0 (-)	1 (7.7%)	0 (-)	1 (6.7%)	3 (8.6%)
HDV	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
HIV-1	1 (5.3%)	0 (-)	0 (-)	0 (-)	1 (2.9%)
HIV-2	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
PIII	15 (78.9%)	10 (76.9%)	20 (76.9%)	11 (73.3%)	13 (37.1%)
ANA	2 (10.5%)	3 (23.1%)	12 (46.2%)	4 (26.7%)	9 (25.7%)
AMA	0 (-)	0 (-)	0 (-)	0 (-)	2 (5.7%)
AM2	1 (5.3%)	0 (-)	3 (11.5%)	1 (6.7%)	3 (8.6%)
ASM	0 (-)	0 (-)	1 (3.8%)	1 (6.7%)	0 (-)
αFP	3 (15.9%)	0 (-)	2 (7.7%)	0 (-)	1 (2.9%)
CA50	3 (15.9%)	5 (38.4%)	1 (3.8%)	0 (-)	2 (5.7%)

<sup>a</sup>AMA, anti-mitochondrial antibodies >1:10; AM2, anti-M2, antibodies >10 units/mL; ANA, antinuclear antibodies >1:10; ASM, anti-smooth muscle antibodies >1:10; CA50, monoclonal anti-CA50 antigen (RIA-gnost®, Behring, Mannheim, Germany) >normal level; αFP, α-fetoprotein >normal level; PIII, procollagen III peptides >normal level.

**Table 5. Prevalence of immunological markers in patients with liver disease and in healthy blood donors**

Markers <sup>a</sup>	Patients with liver disease	Blood donors
PIII	45/84 (53.5%)	51/81 (63.0%)
CA50	72/84 (85.7%)	3/84 (3.5%)
α-Fetoprotein	22/84 (26.2%)	3/81 (3.7%)
ANA	63/84 (75.0%)	24/65 (36.9%)
AMA	19/84 (22.6%)	3/65 (4.6%)
AM2	19/84 (22.6%)	8/50 (16.0%)
ASM	6/84 (7.1%)	1/65 (1.5%)

<sup>a</sup>For explanation, see footnote to Table 4.

**Table 6. Correlation between hepatocellular carcinoma and anti-hepatitis C antibody or hepatitis B surface antigen**

	Total	Anti-hepatitis C <sup>b</sup>		Hepatitis B surface antigen <sup>c</sup>	
		Present	Absent	Present	Absent
Hepatocellular carcinoma <sup>a</sup>					
Present	14	4 (28.6%)	10 (71.4%)	9 (64.3%)	5 (35.7%)
Absent	62	11	51	13	49
Total	76	15	61	22	54

<sup>a</sup>All patients with carcinoma had at least one marker of hepatitis B virus infection.

<sup>b</sup>Correlation between carcinoma and anti-hepatitis C:  $\chi^2=0.8455$ ,  $P=0.36$ .

<sup>c</sup>Correlation between carcinoma and hepatitis B surface antigen:  $\chi^2=10.42$ ,  $P=0.0012$ .

clinical or histological correlation with an autoimmune liver disease be found. α-Fetoprotein was significantly commoner in liver disease patients and children up to 5 years old than in controls.

### Cirrhosis

HBsAg was present in 47% of patients with cirrhosis, somewhat fewer than patients with HCC. Liver cirrhosis

**Table 7. Correlation between liver cirrhosis and hepatitis C virus or hepatitis B surface antigen**

	Total	Hepatitis C virus		Hepatitis B surface antigen	
		Present	Absent	Present	Absent
Cirrhosis					
Present	19	8 (53%)	11 (18%)	9 (41%)	10 (19%)
Absent	57	7 (47%)	50 (82%)	13 (59%)	44 (81%)
Total	76	15	61	22	54

was associated in 42% with anti-HCV, indicating a combined infection with HBV and HCV (Table 7).

Cirrhosis of the liver was correlated with both HCV infection ( $\chi^2=8.0$ ;  $P=0.0047$ ) and the presence of HBsAg ( $\chi^2=4.18$ ;  $P=0.041$ ).

All patients with liver cirrhosis, HCC, and chronic active or persistent hepatitis had at least one serum marker of HBV infection, and 53% (10) of the patients with liver cirrhosis had evidence of a combined infection with HBV and HCV, while 42% (8) of them were HBsAg negative.

Five patients with HCC (38%) showed evidence of combined infection with HBV and HCV, and 5 had markers of HBV infection only.

Of the 7 patients with chronic active and the 3 with persistent hepatitis, 4 and 1, respectively, had markers of both HBV and HCV infection, while 3 and 2, respectively, had markers of HBV infection only.

### Discussion

Although HAV and HBV had infected virtually every adult and a substantial number of the juvenile persons in our study, about one-third of the patients with liver disease were also affected by some other hepatic disease (Table 1).

Ultrasound proved to be particularly useful in the visualization of focal hepatic lesions, although differentiation between amoebic liver abscess, HCC or malignant lymphoma can be very difficult (MISSALEK, 1992). Immediate puncture and aspiration can enable a rapid diagnosis. Thus abdominal ultrasound is an efficient screening diagnostic tool, while laparoscopy is even more helpful in making a definite diagnosis, confirmed by histology.

Clinical signs of chronic liver disease, common among Caucasians, such as spider naevi, palmar erythema, loss of abdominal hair, and testicular atrophy are rarely seen in Black Africans (LEEVEY *et al.*, 1977). Variceal haemorrhage, severe disorders of coagulation and portosystemic encephalopathy were not observed in this survey. Elevation of γ-glutamyltransferase in serum was the most frequently abnormal liver function test. Nevertheless, its sensitivity of only 67% among liver disease patients is too low for screening purposes. Bleeding and coagulation time proved also to be of little help in evaluating diffuse parenchymatous liver disease.

Although a substantial number of HBV infections probably occurs in the perinatal period, anti-HBc posi-

tivity steadily increases in the first 15 years of life (WHO 1991). The source of infection during this period is not clear. Horizontal spread among small children, skin piercing as a traditional treatment or custom (scarification, circumcision), and early sexual activity may all play a role (NDUMBE, 1992; KAO *et al.*, 1993; NDUMBE & SKALSKY, 1993).

The HCV infection rate was comparatively low in the control population, especially in childhood, the time at which most blood transfusions are given for severe, malaria-associated anaemia. Concomitant HCV infection was, however, present in 18% of our patients with liver disease. There was some evidence that the prevalence of anti-HCV antibodies increased with age: 3 of 35 subjects aged over 25 years had anti-HCV IgG, compared with 2 of 73 of those aged 25 years or less (Table 4). This is in agreement with the marked prevalence of anti-HCV in persons over 40 years of age reported in a recent survey of another rural forest area in Cameroon (LOUIS *et al.*, 1994).

Given the high rate of HBV infection in controls and the three-fold increases in prevalence of HCV infection in liver disease patients (a seven-fold increase in cirrhosis) compared to controls, it may be speculated that HCV infection is of considerable importance in the development of chronic liver pathology. The mode of infection with HCV in our population is uncertain. Blood transfusions do not seem to play a major role.

There was a significant positive correlation between liver cirrhosis, HCC and HBsAg, and between liver cirrhosis and anti-HCV, but only a weak positive correlation between HCV infection and HCC.

A high frequency of positive autoantibodies, especially ANA (speckled pattern), has been described previously in regions endemic for falciparum malaria (BOONPUCKNAVIG & EKAPANYAKUL, 1984; see also ACETI *et al.*, 1990). The autoantibodies, however, might also be markers of an autoimmune process, triggered by a previous hepatitis virus infection (LENZI *et al.*, 1991; VENTO *et al.*, 1991). Histologically and clinically, however, we found neither chronic autoimmune hepatitis nor primary biliary cirrhosis in our survey, despite the rather high prevalence of ANA and AM2 autoantibodies among liver disease patients.

The prevalence of a positive rapid plasma reagin test (RPR) for syphilis (17%) and the HCV prevalence of 6% in pregnant women (Table 3) were relatively high. Similar results have been reported from the adult population of Yaoundé, the capital of Cameroon, where the sera were tested by *Treponema pallidum* haemagglutination (TPHA), a more specific antibody test for syphilis (NDUMBE, 1992), and for hepatitis C antibodies (MENCARINI, 1991).

Whether there is a relationship between genital ulcer disease and HCV transmission, as there is for HIV-1 transmission, needs further investigation. This was suggested by further observations from Yaoundé, where the seroprevalence of HCV antibodies among prostitutes was about twice that of pregnant women (NDUMBE & SKALSKY, 1993) and by the fact that, in our cohort, the seroprevalence of HCV antibodies among pregnant women with a positive RPR was about twice that observed in

RPR negative pregnant women.

Positive HIV-1 status seemed not to have an influence on the expression of the different anti-hepatitis antibodies, especially HBsAg and anti-HCV, in our study.

As HBV and HCV seem to play an important role in chronic liver disease in the area we investigated, the prevalence of HBsAg (5.4%) and anti-HCV antibodies (6.1%) in pregnant women, with the risk of vertical transmission (THALER *et al.*, 1992), is of epidemiological importance and supports the introduction of early vaccination against HBV and, in the future, if available, HCV in the Expanded Programme on Immunization (WHO, 1991; NDUMBE & YENSHU, 1992).

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Received 9 November 1994; revised 6 February 1995; accepted for publication 9 February 1995